

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|---|-----------|--|
| (51) International Patent Classification ⁶ : A61K 9/51, 9/16, 47/24, 47/28, 31/70, 38/23, 38/09 | A1 | (11) International Publication Number: WO 99/27918 (43) International Publication Date: 10 June 1999 (10.06.99) |
| (21) International Application Number: PCT/EP98/07664 (22) International Filing Date: 27 November 1998 (27.11.98) (30) Priority Data: MI97A002663 1 December 1997 (01.12.97) IT (71)(72) Applicant and Inventor: GASCO, Maria, Rosa [IT/IT]; Lungo Po Antonelli 207, I-10153 Torino (IT). (74) Agent: GERVASI, Gemma; Notarbartolo & Gervasi S.p.A., Corso di Porta Vittoria, 9, I-20122 Milan (IT). | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |
| (54) Title: MICROPARTICLES FOR DRUG DELIVERY ACROSS MUCOSA AND THE BLOOD-BRAIN BARRIER | | |
| (57) Abstract <p>Pharmaceutical composition comprising microparticles having an average diameter ranging from 40 to 150 nm, consisting of one or more lipids, a drug and, optionally, a steric stabilizer, suitable to the transmucosal passage and to the overcoming of the blood-brain barrier and the blood-cerebrospinal fluid barrier, said microparticles being obtained dispersing in an aqueous medium cooled to 2-4 °C an oil/water or a water/oil/water microemulsion comprising said constituents.</p> | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | ML | Mali | TR | Turkey |
| BG | Bulgaria | HU | Hungary | MN | Mongolia | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MR | Mauritania | UA | Ukraine |
| BR | Brazil | IL | Israel | MW | Malawi | UG | Uganda |
| BY | Belarus | IS | Iceland | MX | Mexico | US | United States of America |
| CA | Canada | IT | Italy | NE | Niger | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NL | Netherlands | VN | Viet Nam |
| CG | Congo | KE | Kenya | NO | Norway | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NZ | New Zealand | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland | | |
| CM | Cameroon | KR | Republic of Korea | PT | Portugal | | |
| CN | China | KZ | Kazakstan | RO | Romania | | |
| CU | Cuba | LC | Saint Lucia | RU | Russian Federation | | |
| CZ | Czech Republic | LI | Liechtenstein | SD | Sudan | | |
| DE | Germany | LK | Sri Lanka | SE | Sweden | | |
| DK | Denmark | LR | Liberia | SG | Singapore | | |
| EE | Estonia | | | | | | |

MICROPARTICLES FOR DRUG DELIVERY ACROSS MUCOSA AND THE BLOOD-BRAIN BARRIER

Prior art

5 An important problem in the field of the administration and the absorption of drugs consists of the difficulty of the passage for some drugs through the intestinal mucosa and of the difficulty of the passage through the blood-brain barrier and the blood-cerebrospinal fluid barrier.

In fact the drugs administered by mouth and destined to pass through the
10 intestinal mucosa find a limitation due to the gastrointestinal pH, to the residence time and to the solubility.

For example biodegradable drugs such as proteins and peptides, and slightly soluble drugs such as some cytostatics are not suitable for this kind of administration.

15 On the other hand drugs as the peptides or the antibiotics such as ampicillin and the anti-tumour drugs such as the cyclophosphamide are not able to pass through the blood-brain barrier.

Several researches on the absorption of the colloidal polymeric particles of polystyrene, polyglycol lactates, polyalkilcyanoacrylates ansacrylates and of
20 polymeric liposomes as carriers of drugs by the gastrointestinal tract after the administration by mouth have been carried out and the passage of said particles through the intestinal mucosa has been proved.

The results of said researches are described for example by A. T. Florence, The Oral Absorption of Micro- and Nanoparticulates:

25 Neither Exceptional, Not Unusual, Pharm. Res. 14, 259 (1997) and by H. Chen, V. Torchilin, R. Langer, Polymerized Liposomes as Potential Oral Vaccine: Stability and Bioavailability, J. Controlled Release. 42, 263 (1996).

However said colloidal particles have the drawback of a very low passage. Moreover the polymers contain traces of solvents and degradation products which
30 are not pharmaceutically acceptable.

Finally the polystyrene is not acceptable because it is not biodegradable.

As far as the preparation of compositions suitable to the passage through the blood-brain barrier is concerned, nanoparticles of polybutylcyanoacrylate containing drugs such as the gentamicin, administered by intravenous way in rats have been used obtaining very partial results. More often one resorts to the implantation of the carriers in the skull in the case of brain cancers (Menei P. et al., Neurosurgery 39, 117 (1996)).

Summary

Pharmaceutical compositions in the form of microparticles suitable to the passage through the intestinal mucosa, the blood-brain barrier and the blood-cerebrospinal fluid barrier have been now found.

Said microparticles have a size ranging from 40 to 150 nm, they are formed by one or more lipids optionally in combination with a steric stabilizer and by a drug. Said microparticles are prepared dispersing in an aqueous medium at 2-4 °C a hot prepared oil/water or water/oil/water microemulsion comprising one or more lipids, a surfactant agent, a cosurfactant agent and optionally a steric stabilizer.

Detailed description of the invention

The characteristics and the advantages of the pharmaceutical compositions in the form of microparticles suitable to the passage through the intestinal mucosa, the blood-brain barrier and the blood-cerebrospinal fluid barrier according to the present invention will be mostly shown during the following description.

Said microparticles are obtained dispersing in an aqueous medium cooled at 2-4 °C an oil/water or water/oil/water microemulsion prepared according to the following description.

The preparation by the oil/water microemulsion is carried out by the following steps:

- a) a mixture consisting of one or more lipids, at least a surfactant agent and at least a cosurfactant agent is warmed at a temperature at least equal to the melting temperature of the lipids;
- b) a mixture consisting of a drug dissolved or dispersed in water and optionally a steric stabilizer, warmed at a temperature at least equal to the temperature of the step a) is added to said mixture of step a), obtaining an oil/water microemulsion;

c) the microemulsion is dispersed in an aqueous medium at 2-4 °C obtaining the microparticles in suspension;

d) the microparticles suspension is washed by an aqueous medium by diafiltration and freeze-dried.

5 The preparation by the water/oil/water microemulsion is carried out by the following steps:

a') a mixture consisting of one or more lipids, at least a surfactant agent and at least a cosurfactant agent is warmed at a temperature at least equal to the melting temperature of the lipids;

10 b') a mixture consisting of a drug dissolved or dispersed in water warmed at a temperature at least equal to the temperature of the step a') is added to the mixture of step a') obtaining a water/oil microemulsion;

c') a mixture consisting of water, at least a surfactant agent and at least a cosurfactant agent and optionally a steric stabilizer warmed at a temperature at
15 least equal to the temperature of the step a') is added to said microemulsion obtaining the water/oil/water microemulsion;

d') said water/oil/water microemulsion is dispersed in an aqueous medium at 2-4 °C obtaining the microparticles in suspension;

e') the microparticles suspension is washed by water by diafiltration and freeze-
20 dried.

The amount of water used in the steps c) and d') is ranging from 5 to 200 volumes per volume of the respective microemulsion.

The obtained microparticles have an average diameter ranging from 40 to 150 nm and preferably from 60 to 100 nm and a polydispersion index ranging from 0.15 to
25 0.28.

The lipids used in the preparation of the microparticles according to the present invention are selected from the group consisting of the stearic acid, the palmitic acid, the triglycerides, the diglycerides and the monoglycerides.

The surfactant agents are selected among soy-bean phosphatidylcholine, dioleoyl
30 phosphatidylcholine, dipalmitoyl phosphatidylcholine, hydrogenated soy-bean phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine.

The cosurfactant agents are selected among ethanol, propanol, isopropanol, butanol, sodium taurocholate, sodium glycocholate, propylene glycol, butyric acid and benzoic acid.

The possible steric stabilizer is selected among dipalmitoyl phosphatidyl
5 ethanolamine-PEG, PEG-stearate, the esters of the fatty acids from the myristic acid to the docosanoic acid with methyl ether PEG, the diacylphosphatidyl ethanolamines esterified with methyl ether PEG and the polylactates and the polyglycolactates esterified with methyl ether PEG.

The methyl ether PEG has preferably a molecular weight ranging from 750 to
10 2000.

The washing by diafiltration of the steps d) and e') has the aim to remove the surfactant agent, the cosurfactant agent and the possible drug not included in the lipid so that the final composition of the microparticles results:

■ lipids, from 80 to 99% by weight,

15 ■ drug, from 1 to 20% by weight,

or:

■ lipids, from 75 to 98.5% by weight,

■ steric stabilizer, from 0.5 to 15% by weight,

■ drug, from 1 to 10% by weight.

20 The microparticles according to the present invention are successfully used in the preparation of the compositions suitable to the administration by mouth for the transmucosal absorption directed towards the lymphatic system and for the intravenous administration for the overcoming of the blood-brain barrier and the blood-cerebrospinal fluid barrier.

25 The compositions containing the steric stabilizer are particularly suitable for the intravenous administration.

Forms suitable to the administration by mouth are aqueous dispersions, having a microparticle content ranging from 20 to 200 mg/ml.

Forms suitable to the intravenous administration are aqueous dispersions having
30 a microparticle content ranging from 30 to 150 mg/ml.

Drugs particularly suitable for the transmucosal way are cytostatic drugs used for

the therapy of the lymphomas such as methotrexate, hydaryubicin, cyclophosphamide, vincristine and vinblastine, antibiotics such as the gentamicin and peptides such as calcitonin, LHRH and analogous ones.

Drugs particularly suitable for the passage of the blood-brain barrier and the
5 blood-cerebrospinal fluid barrier are the peptides such as LHRH and analogous ones, enkephalins, antibiotics such as ampicillin and gentamicin and anti-tumour drugs such as the cyclophosphamide and derivatives, carmustine and carboplatinum.

As far as the transmucosal transport is concerned we have the advantage that the
10 transport by lymphatic way avoids the first passage through the liver, it allows the administration by mouth of drugs, such as lipids, for which such administration is not always possible and it allows the treatment of the lymphatic system cancers.

As far as the transport through the blood-brain barrier and the blood-cerebrospinal fluid barrier is concerned, the compositions according to the present
15 invention allow the passage of drugs included in the carriers which normally do not pass or pass in an insufficient amount to obtain a suitable therapeutic effect.

Pharmacological Experimentation

Radio-labelled microparticles have been prepared acting according to the Example 3, reported below, and adding a solution of ¹³¹iodoheptadecanoic acid
20 to the warm oil/water microemulsion.

The experimentation has been carried out in male albin rats of a Wistar strain (Charles River-Italy) having a weight equal to 550-650 g.

Doses equal to 10 mg/kg (about 40 μ Ci) of an aqueous dispersion of microparticles have been administered at the duodenal level to different groups of
25 rats.

Samples of lymph from the thoracic duct and blood from the jugular vein have been taken at different times.

The data are reported in the Table 1 wherein the radioactivity percentage is reported with respect to the dose administered per gram of lymph and per gram
30 of blood as a function of the time.

TABLE 1

Average values of radioactivity percentage of the administered dose per gram of lymph (A) or of blood (B) determined in time by gamma counting.

| Time after the administration | A radioactivity%/Lympha g. | B radioactivity%/Blood g. |
|----------------------------------|-------------------------------|------------------------------|
| 30' | 1 | 0.09 |
| 60' | 1.5 | 0.08 |
| 90' | 3 | 0.08 |
| 120' | 9 | 0.07 |
| 150 | 8 | 0.07 |
| 180 | 7.5 | 0.07 |

5

The same dispersion has been administered by intravenous way to the rats (10 mg/kg) (about 30 μ Ci). The passage through the blood-brain barrier is pointed out by the presence in the liquor of about 5% of the total radioactivity after 10 minutes from the administration.

- 10 For illustrative aim the following Examples of microparticles preparations according to the present invention are reported.

EXAMPLE 1

- 15 a) A mixture consisting of 180 mg of stearic acid, 92 mg of phosphatidylcholine, 12 mg of dioleoyl phosphatidylcholine, 72 mg of butanol and 16 mg of butyric acid is warmed at 70 °C.

b) 28 mg of an aqueous solution of LHRH (40 mg/ml) warmed at 70 °C, are added under stirring to the mixture of the step a) obtaining a water/oil microemulsion.

- c) A mixture consisting of 274.4 mg of water, 16.8 mg of soy-bean phosphatidylcholine, 20.8 mg of butanol, 32.4 mg of sodium taurocholate and 4.0
20 mg of butyric acid, warmed at 70 °C, is added under stirring to 52 mg of the water/oil microemulsion of the step b) obtaining a water/oil/water microemulsion.

d) The water/oil/water microemulsion of the step c) is dispersed in a ratio equal to 1:10 in water at a temperature equal to 2-3 °C obtaining the microparticles in an

aqueous dispersion.

e) The aqueous dispersion of the step d) is washed three times with water by diafiltration and subsequently it is freeze-dried.

The following results are obtained:

- 5 ■ drug incorporated in the microparticles: 80%;
- average diameter of the microparticles: 96 nm;
- polydispersion index: 0.23.

EXAMPLE 2

A mixture consisting of 273.6 mg of water, 16.4 mg of phosphatidylcholine, 20.0
10 mg of butanol, 32.0 mg of sodium taurocholate, 2.4 mg of butyric acid and 2.8 mg
of dipalmitoyl phosphatidyl ethanolamine-PEG (steric stabilizer), warmed at 70 °C,
is added under stirring to 52.0 mg of the water/oil microemulsion of the step b) of
the Example 1.

The preparation is completed according to the steps d) and e) of the Example 1.

15 The following results are obtained:

- drug incorporated in the microparticles: 78%;
- average diameter of the microparticles: 100 nm;
- polydispersion index: 0.24.

EXAMPLE 3

20 a) A mixture consisting of 24 mg of stearic acid and 6.0 mg of monostearine is
warmed at 70 °C;

b) a mixture consisting of 6.0 mg of gentamicin, 56.0 mg of sodium taurocholate,
28.0 mg of soy-bean phosphatidylcholine and 280.0 mg of water, warmed at 70
°C, is added under stirring to the mixture of the step a) obtaining an oil in water
25 microemulsion.

The microemulsion obtained in the step b) is then treated as described in the
steps d) and e) of the Example 1.

The following results are obtained:

- gentamicin incorporated in the microparticles: 80 %;
- 30 ■ average diameter of the microparticles: 105 nm;
- polydispersion index: 0.22.

EXAMPLE 4

a) A mixture consisting of 210.0 mg of stearic acid, 10.0 mg of monostearine, 62.0 mg of soy-bean phosphatidylcholine, 80.0 mg of butyric acid and 6.0 mg of butanol is warmed at 70 °C.

5 b) 36.0 mg of an aqueous solution of calcitonin (2 mg/ml) warmed at 68 °C are added to the mixture of the step a) obtaining a water/oil microemulsion.

c) A mixture consisting of 214.4 mg of water, 16.8 mg of soy-bean phosphatidylcholine, 16.4 g of butyric acid, 8.0 g of butanol and 32.4 g of sodium taurocholate, warmed at 70 °C, is added under stirring to 52.0 mg of the water/oil
10 microemulsion of the step b), obtaining a water/oil/water microemulsion.

The preparation is then completed working according to the steps d) and e) of the Example 1.

The following results are obtained:

- incorporation of the calcitonin: 83 %;
- 15 ■ average diameter of the microparticles: 93 nm;
- polydispersion index: 0.23.

EXAMPLE 5

A mixture consisting of 212.4 mg of water, 16.8 mg of soy-bean phosphatidylcholine, 16.4 mg of butyric acid, 8.0 g of butanol, 32.4 mg of sodium
20 taurocholate and 8.0 mg of stearic acid-PEG (steric stabilizer) warmed at 68 °C is added under stirring to 52.0 mg of the water/oil microemulsion of the step b) of the Example 4.

The preparation is completed according to the steps d) and e) of the Example 1.

The following results are obtained:

- 25 ■ incorporation of the calcitonin: 70 %;
- average diameter of the microparticles: 102 nm;
- polydispersion index: 0.23.

CLAIMS

- 1 1. Use of microparticles consisting of one or more lipids, a drug and optionally a
2 steric stabilizer for the preparation of compositions suitable to the transmucosal
3 passage and to the overcoming of the blood-brain barrier and the blood-
4 cerebrospinal fluid barrier.
- 1 2. Use as claimed in claim 1, characterized in that said microparticles have an
2 average diameter ranging from 40 to 150 nm and a polydispersion index ranging
3 from 0.15 to 0.28.
- 1 3. Use as claimed in claim 1, characterized in that said microparticles consist of
2 one or more lipids in an amount ranging from 80 to 99% by weight and of a drug in
3 an amount ranging from 1 to 20% by weight.
- 1 4. Use as claimed in claim 1, characterized in that said microparticles consist of
2 one or more lipids in an amount ranging from 75 to 98.5% by weight, of a steric
3 stabilizer in an amount ranging from 0.5 to 15% by weight and of a drug in an
4 amount ranging from 1 to 10% by weight.
- 1 5. Use as claimed in claim 1, characterized in that said compositions consist of
2 aqueous dispersions having a content of said microparticles ranging from 20 to
3 200 mg/ml.
- 1 6. Use as claimed in claim 1, characterized in that said lipids are selected from the
2 group comprising the stearic acid, the palmitic acid, the triglycerides, the
3 diglycerides, the monoglycerides.
- 1 7. Use as claimed in claim 1, characterized in that said steric stabilizer is selected
2 from dipalmitoyl phosphatidyl ethanolamine-PEG, PEG-stearate, esters of the fatty
3 acids from the myristic acid to the docosanoic acid with methyl ether PEG,
4 diacylphosphatidyl ethanolamines esterified with methyl ether PEG and
5 polylactates and polyglycolactates esterified with methyl ether PEG.
- 1 8. Use as claimed in claim 1, characterized in that said drug is selected from the
2 group consisting of methotrexate, hydarubicin, cyclophosphamide, vincristine,
3 vinblastine, gentamicin, calcitonin, LHRH, enkephalins, ampicillin, gentamicin,
4 carmustine, and carboplatinum.
- 1 9. Use as claimed in claim 1, characterized in that said microparticles are obtained

2 dispersing, in an aqueous medium cooled at 2-4 °C, an oil in water or a
3 water/oil/water microemulsion warmed at a temperature near the melting
4 temperature of the lipids, washing with water by diafiltration and freeze-drying.

1 10. Use as claimed in claim 9, characterized in that said microemulsions comprise
2 at least a surfactant agent and at least a cosurfactant agent.

1 11. Compositions suitable to the transmucosal passage and to the overcoming of
2 the blood-brain barrier and the blood-cerebrospinal fluid barrier, consisting of
3 aqueous dispersions of microparticles having an average diameter ranging from
4 40 to 150 nm and a polydispersion index ranging from 0.15 to 0.28, said
5 microparticles consisting of one or more lipids, a drug and, optionally a steric
6 stabilizer.

1 12. Compositions as claimed in claim 11, characterized in that said microparticles
2 are present in said aqueous dispersions in an amount ranging from 20 to 200
3 mg/ml.

1 13. Compositions as claimed in claim 11, suitable to the administration by mouth
2 and to the intravenous administration.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/07664

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K9/51 A61K9/16 A61K47/24 A61K47/28 A61K31/70
A61K38/23 A61K38/09

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|-----------------------|
| X | EP 0 167 825 A (RENTSCHLER ARZNEIMITTEL) 15 January 1986 see abstract see page 4, line 6 - page 5, line 31 see page 11, line 17 - line 23 see page 12, line 18 - line 21 see page 14, line 1 - line 8 see examples 7-15 see claims 1-13 --- -/-- | 1-13 |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

21 April 1999

Date of mailing of the international search report

12/05/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3015

Authorized officer

Taylor, G.M.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 98/07664

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|---|-----------------------|
| X | KREUTER J., ET AL.: "Passage of peptides through the blood brain barrier with colloidal polymer particles (nanoparticles)" BRAIN RESEARCH, vol. 674, 1995, pages 171-174, XP002099976 see abstract see page 171 - page 174 see figure 1 --- | 1-13 |
| X | WO 95 05164 A (WESTESEN KIRSTEN :SIEKMANN BRITTA (SE)) 23 February 1995 see abstract Y see claims 1-62 --- | 1-7,9-13 |
| X | EP 0 526 666 A (GASCO MARIA ROSA) 10 February 1993 see abstract see column 2, line 19 - line 24 see column 2, line 26 - column 3, line 36 see example 2 Y see claims 1-15 --- | 1-7,9-13 |
| X | DE 41 31 562 A (MEDAC KLINISCHE SPEZIALPRAEP) 25 March 1993 see abstract see page 2, line 1 - line 5 see page 3, line 20 - page 4, line 29 see page 4, line 36 - line 63 see examples 1-6 Y see claims 1-16 --- | 1-7,9-13 |
| E | WO 98 56362 A (GASCO MARIA ROSA) 17 December 1998 see abstract see page 1, line 16 - line 22 see page 3, line 14 - line 17 see page 4, line 17 - line 21 see examples 1-4 see claims 1-17 ----- | 1-13 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 98/07664

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| EP 0167825 A | 15-01-1986 | DE 3421468 A | 19-12-1985 |
| | | AT 55243 T | 15-08-1990 |
| | | JP 61056122 A | 20-03-1986 |
| | | US 4880634 A | 14-11-1989 |
| WO 9505164 A | 23-02-1995 | DE 4327063 A | 16-02-1995 |
| | | AU 7392694 A | 14-03-1995 |
| | | EP 0711151 A | 15-05-1996 |
| | | JP 9502963 T | 25-03-1997 |
| EP 0526666 A | 10-02-1993 | AT 136774 T | 15-05-1996 |
| | | DE 69118880 D | 23-05-1996 |
| | | DE 69118880 T | 07-11-1996 |
| | | ES 2089066 T | 01-10-1996 |
| | | US 5250236 A | 05-10-1993 |
| DE 4131562 A | 25-03-1993 | AT 135567 T | 15-04-1996 |
| | | AU 672177 B | 26-09-1996 |
| | | AU 2561592 A | 27-04-1993 |
| | | CA 2119253 A,C | 01-04-1993 |
| | | CZ 9400596 A | 13-07-1994 |
| | | DE 59205783 D | 25-04-1996 |
| | | DK 605497 T | 05-08-1996 |
| | | WO 9305768 A | 01-04-1993 |
| | | EP 0605497 A | 13-07-1994 |
| | | ES 2085035 T | 16-05-1996 |
| | | GR 3019750 T | 31-07-1996 |
| | | HU 75165 A | 28-04-1997 |
| | | JP 2683575 B | 03-12-1997 |
| | | JP 6510772 T | 01-12-1994 |
| | | KR 141504 B | 01-06-1998 |
| WO 9856362 A | 17-12-1998 | IT MI971385 A | 14-12-1998 |
| | | AU 7915898 A | 30-12-1998 |